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Significance: This study highlights potential differences that can arise in gaze position estimates from 1st Purkinje image-based eye trackers based on how individual Hirschberg ratios are calculated.

Purpose: To evaluate the accuracy and repeatability of eccentric-viewing, prism-based and theoretical techniques that are routinely used to calibrate Hirschberg Ratio (HR) in 1st Purkinje image-based eye trackers.

Methods: Hirschberg ratios of 28 UK participants (18-40 years) were obtained using the PlusOptix PowerRef3 photorefractor and eye tracker. In the gold-standard eccentric viewing technique, participants viewed eccentric targets (±12°, 4° steps) at 2m. In the prism-based technique, 4-16ΔD base-out and base-in prisms were placed in 4ΔD steps before an eye occluded with an infrared filter; the fellow eye fixated a target at 1m. Each participant's HR was calculated as the slope of the linear regression of the shift in Purkinje image, relative to the pupil centre, for each target eccentricity or induced prism power. Theoretical HR was calculated from the participant's corneal curvature and anterior chamber depth measures. Data collection was repeated on another visit using all three techniques to assess repeatability. Data were also obtained from an Indian cohort (n=30,18–40 years) using similar protocols.

Results: HR ranged from 10.61-14.63°/mm (median: 11.90°/mm) in the eccentric viewing technique. The prism-based and theoretical techniques demonstrated inaccuracies of 12% and 4% relative to the eccentric viewing technique. The 95% limits of agreement of intra-subject variability ranged from ±2.00°/mm, ±0.40°/mm, and ±0.30°/mm for the prism-based, eccentric viewing and theoretical techniques (P>.05). Intraclass correlation coefficients (95%CI) were 0.99 (0.98-1.00) for eccentric, 0.99 (0.99-1.00) for theoretical and 0.88 (0.74-0.94) for prism-based techniques. Similar results were found for the Indian cohort.

Conclusion: The prism-based and theoretical techniques both demonstrated relative inaccuracies in measures of Hirschberg ratio, compared to the eccentric viewing technique. The prism-based technique exhibited the poorest repeatability.

Keywords: Anterior chamber depth, corneal curvature, eye tracker, gaze position, Hirschberg ratio, Purkinje-image, repeatability, variability

Video-based eye trackers are increasingly used in research laboratories and clinical settings to obtain quantitative estimates of gaze position.[1-3] This is a valuable measure in studies investigating the biology of eye movements or those that use movements as an objective biomarker for assessing cognition[4, 5] or tracking disease progression.[6] These trackers use specific landmarks (e.g. 1st Purkinje image, limbus, pupil margin and blood vessel configuration) to rapidly and non-invasively track gaze position.[7, 8] Of the variety of eye trackers commercially available, those using the relative location of the 1st Purkinje image and entrance pupil center (i.e. virtual image of the anatomical pupil as seen through the cornea and anterior chamber[9]) as landmarks are of specific interest to the present study. A proportionality constant or calibration factor – the Hirschberg ratio is used in these trackers to directly convert the millimetre separation between the Purkinje image and pupil center into angular units of gaze position in degrees or prism dioptres (ΔD).[10, 11] Accuracy of gaze position estimates obtained by these trackers, relative to a gold-standard, depends on the accuracy of this calibration factor and on the angle kappa (i.e. the angle between the pupillary axis and visual axis).[12,3] Previous studies have reported a large inter-subject variability in Hirschberg ratio between 7 to 16°/mm or (12 to 28 ΔD/mm),[3, 9, 12, 13] and consequently, using a population-average Hirschberg ratio as the calibration factor (e.g. 11.8°/mm as used by the PlusOptix PowerRef3 device used in this study[12]) could result in inaccurate estimation of eye position. Using an individual's Hirschberg ratio as the calibration factor therefore enhances the accuracy of gaze position estimates determined by these eye trackers.[12]

Three techniques have been described in the literature to measure the individual Hirschberg ratio.[12-14] In the eccentric viewing technique, Hirschberg ratio is measured by asking participants to fixate on targets placed before them at known angular eccentricities and measuring the separation between the Purkinje image and pupil center for each of these positions using the eye tracker's high-resolution camera.[12] The reciprocal of the slope of a linear regression fit of the measured separation between Purkinje image and pupil center against the corresponding target eccentricity gives the Hirschberg ratio in degrees or prism dioptres per millimetre.[7, 11, 15] In the theoretical technique, Hirschberg ratio is derived from biometric measures of the eye's corneal curvature and anterior chamber depth using basic geometric formulae derived by Brodie.[7, 12] Finally, the prism-based technique involves the use of prism of known base powers to change the separation between Purkinje image and pupil center and derives Hirschberg ratio by calculating the reciprocal of the slope of a linear regression fit of the measured separation between these landmarks against a range of induced prism power.[14, 16] The fundamental characteristics, and practical advantages and disadvantages of all three techniques are presented in Table 1.

Table 1 about here

The utility of a given technique for measuring Hirschberg ratio in a clinical or research setting depends on how successfully the technique can be implemented, how accurate the estimates of eye positions are using this technique and how variable are the eye position estimates over repeat measurements using this technique for the subject group. While some studies have investigated the accuracy and repeatability of the eccentric viewing and the theoretical techniques and the agreement between them,[3, 11, 12, 17] there is no such information available for the prism-based technique. There are also no published data describing how the three techniques compare with each other. Therefore, the overall aim of this study was to determine the accuracy, repeatability and agreement of Hirschberg ratios obtained using each one of these three techniques.

Materials and methods

Adult subjects aged 18 – 40 years were recruited from staff and students of Ulster University (UU), Coleraine, Northern Ireland, UK (n=28). All subjects that participated in the study had a presenting distance visual acuity of 20/20 or better and were either emmetropic (-0.25D to +1.00D spherical equivalent refractive error) or myopic (-0.50 to -6.00D spherical equivalent refractive error) for the fixated eye (Table 2). Subjects were required to have refractive errors limited to <1.00D anisometropia, and no more than 0.75DC of astigmatism. Subjects were corrected using soft contact lenses as measurement of lens calibration was also being undertaken as a part of a different study (Table 2). These soft contact lenses conformed to the shape of the cornea and produced minimal movement during measurement. None of the subjects presented with any ocular pathology or binocular vision anomaly. Subjects with amblyopia, pupil diameters <3.00mm (below the operational range of the device) in a dimly lighted room, who could not maintain steady fixation or had an excessive blink rate were excluded.

Data from a parallel study with small variations in experimental protocol and participant cohort were collected from the Brien Holden Institute of Optometry and Vision Sciences, LV Prasad Eye Institute (LVPEI), Hyderabad, India to determine the generality of the study outcomes in the UU cohort. Table 2 summarises the participant characteristics in both cohorts. The study was conducted in accordance with the tenets of the Declaration of Helsinki and commenced after approval by the local Research Ethics Committee at UU and the Institutional Review Board at LVPEI.

Gaze position data were obtained using the PowerRef 3™ (Nuremberg, Germany) at a sampling frequency of 50Hz for the prism-based calibration and the eccentric viewing calibration techniques. The device is designed to capture dynamic measurements of the eye's refractive power at the pupil plane using eccentric photorefraction technique.[18] To do so, the device is fitted with an array of infrared light emitting diodes adjacent to the camera aperture, and this arrangement produces a crisp 1st Purkinje image, which can be compared to the position of the pupil center in the vertical and horizontal meridian to determine respective gaze positions. Additional technical information about the photorefractor used in this study can be found at https://plusoptix.com/images/support-downloads/powerref3-specifications-usa.pdf. The use of different versions of eccentric photorefractors for eye movement research has been previously reported,[12, 14, 16, 19] and more research is being conducted with these instruments. Therefore, this study used the PowerRef 3™ as a model instrument of 1stPurkinje image-based eye trackers for investigating the repeatability of three gaze position calibration techniques, using eccentric viewing technique as the reference gold-standard. The set-up for these techniques is described below for the UU data collection. The LVPEI data collection is broadly similar, but Table 2 lists the variations between the two study sites.

Figure 1 about here

Eccentric viewing technique

In the eccentric viewing technique, subjects fixated with their left eye on a series of Maltese cross targets arranged linearly at 2m while the right eye was occluded. The entire target array, from left to right, produced eccentricity of ±12°, with each target separated from the adjacent by 4°. These target eccentricities were all within the ±25°range described by Brodie, where the millimetre separation between the Purkinje image and pupil center varies linearly with angular eccentricity.[13] Beyond this range, the separation between these two landmarks varies in a sinusoidal manner with angular eccentricity.[13]

Prism-based technique

In the prism-based calibration technique, the dominant eye fixated on a Maltese cross target at 1m while the fellow non-dominant eye was occluded using an Optcast long pass infrared transmitting filter (Edmund Optics™, NT43-954). This filter blocks virtually all the visible wavelengths while allowing infrared light from the camera to pass through it. Prisms from 0ΔD to16ΔD in 4ΔD steps were held before the occluded eye in a base-in and base-out sequence, at a vertex distance of 10–14 mm for at least four seconds each. This experimental set-up helped to eliminate any compensatory vergence eye movements from the fellow eye that might contaminate the eye position calibration results. Prism powers were converted into degrees using the formula: where degrees = arctan (prism dioptres/100). The prism powers used here thus corresponded to target eccentricities of ±9.09°, in steps of 2.29°.

Table 2 about here

Gaze position calibration measurements in both prism-based and eccentric viewing techniques were made in a dimly lit room. Data analysis was performed using custom written software in Matlab®. Raw PowerRef 3™ data were processed, removing blinks and extraneous data outside of the instrument working range. Eye movement data were plotted against time and scrutinised for a section of stable gaze, and two seconds worth of data (~100 samples) from each prism power or target position was selected and averaged.[14, 16] These averaged gaze positions were plotted against the corresponding prism power and target eccentricity in prism-based and eccentric viewing techniques, respectively. Linear regression analysis was performed to obtain the eye position calibration slope. The slope of this linear regression equation provided an estimate of the subject's Hirschberg ratio. The calibration slope obtained from these two techniques is a unitless quantity describing the change in eye position recorded by the PowerRef 3™ for a unit change in target eccentricity or prism power. The actual Hirschberg ratio of the individual will be equal to that used by the PowerRef 3™ divided by the calibration slope of that individual obtained using these techniques. In other words, an eye position calibration slope that is equal to unity indicates an Hirschberg ratio of 11.8°/mm (i.e. equal to the population average value used by the machine). Eye position calibration slopes greater than unity correspond to Hirschberg ratios <11.8°/mm (smaller than population average value) while calibration slopes smaller than unity correspond to Hirschberg ratios >11.8°/mm (larger than population average value).

<u>Theoretical technique</u>

The theoretical calibration technique was based on the geometric optics model described by Brodie, which posits that the Hirschberg ratio of the individual varies with their anterior corneal curvature and the anterior chamber depth.[7] The procedure of obtaining Hirschberg ratio using the theoretical technique is explained in detail by Jagini et al.[12] Briefly, the average of three measurements of horizontal corneal curvature and anterior chamber depth of each subject was obtained using the Zeiss IOL Master™. These values were used to theoretically predict the Hirschberg ratio using the regression equation previously described by Jagini et al.[12] All theoretical calculations were performed using custom software in Matlab®. Data from the left eye are presented for the theoretical technique to allow for comparison with the other techniques.

Repeatability of Hirschberg ratio obtained with the three techniques was assessed by repeating each technique for a second time within a week of the first measurement.

Data analysis

Representative eye position raw data and the linear regression fits that were derived from these data for the eccentric viewing and the prism-based techniques are shown in Figure 1. There were excellent linear regression fits for both prism-based and eccentric viewing calibration techniques with r^2 value ≥0.90 in all subjects (consistent with previous work), and this was a criterion for inclusion of data.[12] The Kolmogorov-Smirnov test indicated that the corneal curvature, anterior chamber depth and Hirschberg ratio calculated from the slopes were not normally distributed and, therefore, non-parametric statistics described the data. One-way ANOVA was used to assess the mean difference between the three techniques and the post-hoc Scheffe test was used to determine differences between the individual groups while the paired t-test was used to assess mean differences between baseline and repeat measures. The intraclass correlation coefficient (ICC, two-way mixed effects) was used to assess absolute agreement between measures. Results of intraclass correlation test were considered excellent, good, moderate, and poor if >0.90, 0.75 to 0.90, 0.50 to 0.75, and <0.50, respectively.[20] Linear regression analysis was used to determine range effects in Hirschberg ratio. Significance was determined as $P<.05$.

Results

Data collection was successful in all 28 subjects recruited at UU and in all 30 subjects recruited at LVPEI. The data from the UU cohort will be described first followed by a description of the data in the LVPEI cohort. Given the differences in experimental protocols employed in the two study locations, albeit minor, the two datasets are considered as stand-alone entities and no explicit comparison is made between the two datasets. Only general judgments about the accuracy and repeatability of the Hirschberg ratio measurements from the three protocols are made from the data obtained from the two study locations.

Data from the UU cohort

The baseline regression slopes ranged from 0.81 to 1.11 [median (25^{th}-75^{th}interquartile range; IQR) =0.99 (0.91–1.03)] for the eccentric viewing technique, and 0.70 to 1.03 [0.89 (0.79-0.93)] for the prism-based technique (Table 3). The Hirschberg ratios calculated from these slopes ranged from 10.61 to 14.63°/mm [11.90°/mm (11.44–12.97°/mm)] for the eccentric viewing technique and 11.47 to 16.93 °/mm [13.30°/mm (12.74–15.06°/mm)] for the prism-based technique Table 3 and Figure 2A). Baseline corneal curvatures and anterior chamber depth results are presented in Figure 2B. These translated into theoretically derived Hirschberg ratios ranging from 9.84 to 13.44°/mm [11.43°/mm (10.55–11.96°/mm)] (Table 3).

Table 3 and Figure 2 about here

Results of one-way ANOVA test showed an overall statistically significant difference (signed difference) between the mean Hirschberg ratio of all three techniques ($F_{(2,81)}$ = 31.24, P<.0001). Post-hoc test using Scheffe method showed statistically significant differences between the mean Hirschberg ratio of the prism-based and eccentric viewing techniques (-1.63 °/mm, P<.001), between the prism-based and theoretical techniques (-2.54 °/mm, P<.001) and between the eccentric viewing and theoretical techniques (-0.91 °/mm, P=.024), Table 4 (signed difference). However, to determine if the difference between the techniques varied as a function of calculated Hirschberg ratios, the mean absolute difference between the Hirschberg ratios was computed and presented with the mean signed difference (Table 4). The Bland-Altman type plots of the absolute difference between techniques indicated that there was systematic bias in the Hirschberg ratios obtained from one technique, relative to the other (Figures 3A – C). Linear regression analyses were performed on the absolute difference to determine the effect of Hirschberg ratio size on the mean difference. Results of this analysis indicated that the slopes were significantly different from zero between the prism-based and eccentric viewing techniques (Linear regression equation: Y= -4.18 + 0.46X (X= inter-technique average Hirschberg ratio): $F_{(1,26)}$=7.52, P=.01), and between the prism-based and theoretical techniques (Linear regression equation: Y= -5.51 + 0.64X: $F_{(1,26)}$ =6.98, P=.01, (Figures 3A and B show this effect). However, there was no such Hirschberg ratio range effects on the mean difference between the eccentric and theoretical techniques (Linear regression equation: Y= -0.80 + 0.15X: $F_{(1,26)}$ = 0.34, P=.57), (Figure 3C).

To determine the intra-subject variability of the three Hirschberg ratio techniques, each technique was repeated for a second time within a week of the baseline measurement reported (Figure 4A – C). The mean difference (95% LOA) in Hirschberg ratio between the first and second measurements were 0.05°/mm (95% LOA: -0.30 to 0.40°/mm) for the eccentric viewing technique (Figure 4A) and 0.09°/mm (95% LOA: -1.91 to 2.08°/mm) for the prism-based technique (Figure 4B). Median repeat corneal curvature and anterior chamber depth measures for calculating Hirschberg ratio using the theoretical technique was 7.91 (7.80 – 8.29mm) and 3.54 (3.28 – 3.74), respectively. These translated into median Hirschberg ratio of 11.45°/mm (10.45 – 11.93°/mm). The mean intra-subject variability of the theoretically derived Hirschberg ratio was therefore 0.04°/mm [paired t-test (95% LOA: -0.20 to 0.28°/mm)] (Figure 4C). The mean difference between the first and repeat measures of Hirschberg ratio for all three techniques was not significantly different (all P>.05). Moreover, results of intraclass correlation test revealed excellent repeatability in the eccentric viewing and theoretical techniques [0.99(95% CI: 0.98-0.997), P<.001, and 0.99 (95% CI: 0.99-0.998), P<.001, for the eccentric viewing and theoretical techniques respectively]. In the prism-based technique, there was a good agreement between the first and repeat measures 0.88(95% CI: 0.74-0.944), P<.001) (Table 3).

Figures 3 and 4 about here

Data from the LVPEI cohort

The baseline regression slopes ranged from 0.79 to 1.24 [median (25^{th}-75^{th}IQR) = 1.04 (0.98–1.09)] and 0.76 to 1.12 [0.97 (0.91–1.03)] in the eccentric viewing and prism-based techniques, respectively (Table 3). The Hirschberg ratios derived from these regression slopes are shown in Table 3 and Figure 5A. The baseline corneal curvatures and anterior chamber depth results are presented in Figure 5B and the theoretically derived Hirschberg ratios from these values are shown in Table 3. Results of one-way ANOVA test showed an overall statistically significant difference (signed difference) between the mean Hirschberg ratio of all three techniques ($F_{(2,87)}$ = 4.25, P=.01). Post-hoc test using Scheffe method showed significant differences between the mean Hirschberg ratio of the prism-based and eccentric viewing techniques (-0.77 °/mm, P=.03). However, there was no statistically significant difference between the prism-based and theoretical techniques (-0.67 °/mm, P=.06) and between the eccentric viewing and theoretical techniques (0.10 °/mm, P=.93). As before, results of the absolute difference are presented in Bland Altman plots in Figure 6A-C, see also Table 4. There were no Hirschberg ratio range effects on the mean absolute difference between the prism-based and eccentric viewing techniques (Linear regression equation: Y= -2.13 + 0.27X (X= inter-technique average Hirschberg ratio): $F_{(1,28)}$ =2.79, P=.11), and between the prism-based and theoretical techniques (Linear regression equation: Y= -1.52 + 0.20X: $F_{(1,28)}$ = 3.71, P=.06) (Figure 6A&B). However, range effect was observed on the mean difference between the eccentric viewing and theoretical techniques (Linear regression equation: Y= -2.14 + 0.23X: $F_{(1,28)}$ = 4.62, P=.04) (Figure 6C).

Repeatability of the Hirschberg ratio estimate was available only for the prism-based technique in the LVPEI cohort and this data showed a mean difference (95% LOA) in Hirschberg ratioof 0.08°/mm (95% LOA: -1.7 to 1.9°/mm) (P=.63) (Figure 6D), and the intraclass correlation test of absolute agreement between the first and second measures showed good agreement between the two [0.83(95%CI: 0.64-0.92), P<.001] (see Table 3).

Figure 6 about here

Discussion

The essence of calibrating an individual's Hirschberg ratio in 1st Purkinje image-based eye trackers is to reduce the errors in gaze position estimates that may arise while using the population-average Hirschberg ratio.[3, 9, 12] This problem is of real concern to commonly used eye trackers given the large inter-subject variability in Hirschberg ratio that has been reported in the literature.[3, 9, 11, 12] Following the decision to use the subject's own Hirschberg ratio to calibrate the eye tracker for improved accuracy, a second challenge is to determine which calibration technique is to be adopted for this purpose. The performances of three such techniques that have been used previously in the literature –eccentric viewing, prism-based and theoretical – were tested in the present study. To determine the accuracy of a given calibration technique, the values obtained by this technique need to be compared against a "gold-standard" measure. For the present analysis, the eccentric viewing technique is considered as the "gold-standard" technique simply because of its traditional use for calibrating the Hirschberg ratio in most 1st PI-based eye trackers.[3, 21] This technique has also become a "legacy technique" from which the population-average Hirschberg ratio has been derived in previous studies.[3, 21] Moreover, this technique uses angles anchored in space, and it is based on actual eye rotations, thus requires few assumptions to be made for deriving the Hirschberg ratio.

Compared to the eccentric viewing technique, the prism-based and theoretical techniques both demonstrated relative inaccuracies of 12% and 4% respectively in the UU cohort when the median values were compared [see Table 3 for median values: (100- (13.30÷11.90) ×100) = 12%), and (100-(11.43÷11.90) ×100) = 4%)]. In the LVPEI cohort, similar inaccuracies of 7% and 3% were recorded in the prism-based and theoretical techniques respectively. At individual level, these inaccuracies ranged from 6% of underestimation of the Hirschberg ratio to 33% of overestimation in the theoretical technique, and 20% underestimation to 37% overestimation in the prism-based technique in the UU cohort; and 19% underestimation to 13% overestimation, and 23% underestimation to 29% overestimation in the theoretical and prism-based techniques respectively in the LVPEI cohorts.

The present study also demonstrated the over-estimation of Hirschberg ratio by the prism-based technique, relative to both the eccentric-viewing and theoretical techniques (Figure 3A and B). This was particularly significant in the UU cohort, although there were range effects in the bias towards the prism-based technique and with the difference between techniques appearing to increase with an increase in the size of the Hirschberg ratio (Figure 3A and B). However, there was no such range effect between the eccentric viewing and theoretical techniques, and the mean difference between the two was closer to zero compared to the mean differences when the prism-based technique is considered.

In addition to accuracy, the calibration technique's repeatability also needs to be assessed to determine its usefulness in estimating the individual Hirschberg ratio. The theoretical technique demonstrated the least intra-subject variability as the Hirschberg ratio obtained with this technique was repeatable to within ±0.30°/mm 95% LOA in a subject, less than the 0.50°/mm 95% LOA previously reported by Jagini et al.[12] This may be attributed to the more consistent, repeatable measures of corneal curvature and anterior chamber depth (~0.01mm for both measures in this study, less than the 0.08mm reported previously[12, 22, 23]) available in the present study. The Hirschberg ratio obtained using the eccentric viewing technique was repeatable to within ±0.40°/mm 95% LOA in individual subjects; slightly less repeatable than the theoretical technique, but more repeatable than the prism-based technique. Furthermore, there was improved intra-subject repeatability in the eccentric viewing technique in this study than previously reported (1.5 to 3.0 degrees/mm).[21, 24] The use of different fixation targets in the present study compared to previous work could explain the differences in the intra-subject repeatability reported as fixation target characteristics are known to affect the stability of eye movements.[25] Perhaps the use of Maltese cross fixation target in this study minimized micro-eye movements, thereby contributing to enhanced repeatability. The highest intra-subject variability in Hirschberg ratio, with the lowest intraclass correlation coefficient was observed in the prism-based technique. The Hirschberg ratio measured in the prism-based technique was repeatable to within ±2.0°/mm at UU and ±1.9°/mm at LVPEI. There are no previously published data with which to compare these findings, but when compared with the other two techniques in this study, the variability exhibited by the prism-based technique is high (Figure 4, panel B and Figure 6, panel D). Moreover, the lowest intraclass correlation coefficient was recorded in the prism-based technique demonstrating least agreement between the baseline and repeat measures. It is possible that the high variability in Hirschberg ratio exhibited in the prism-based technique is inherent when using prisms for calibration. Variability in Purkinje image displacements which can arise from minimal variance in orientation and/or placement of the prisms before the infrared-occluded eye during repeat measurements will influence the results.[26] Furthermore, variability in a subject's phoria adaptation at different measurement times,[27] could lead to the high variability observed with the prism-based technique. Finally, potential conflicts in fixation between the target presented to the non-occluded eye and the image of the infrared LED's in the occluded eye could lead to additional variability in this technique.

Another way to quantify the precision of a technique, is to compare the intra-subject and inter-subject variability of the technique. If the magnitude of intra-subject variability equals the inter-subject variability produced by the technique, then its usefulness for calibration could be questioned. In the case of theoretical technique, the intra-subject variability was 13% relative to the inter-subject variability [see Table 3 UU section, for a -0.20 to 0.28°/mm of intra-subject variability, expressed as percentage of its inter-subject variability (9.84 to 13.44°/mm): (0.48÷3.6 × 100) =13%]. Similarly, the eccentric viewing technique exhibited 17% variability of the inter-subject value. However, the prism-based technique exhibited 73% variability in both cohorts relative to the inter-subject variability [e.g. for a -1.91 to 2.08°/mm in the UU cohort, expressed as percentage of its inter-subject variability (11.47 to 16.93°/mm): (3.99÷ 5.46 × 100) = 73%]. From these data, it is evident that the prism-based technique exhibited the greatest variability relative to the other two.

In the present study, data from two laboratories that evaluated three gaze position calibration techniques using similar (but not identical) protocols are presented together in one publication. The differences in protocols include: 1) the assessment of agreement between the eccentric viewing and prism-based techniques in the UU data is limited by the fact that the non-dominant eye was used in the prism-based technique, whereas the left eye was used in eccentric viewing technique (Table 2). However, subjects were all binocularly normal, and wore optical correction during assessments, which would have minimized any possible effects. Additionally, there was a high intraclass correlation between the two ocular biometric measures of the two eyes [0.99(95% CI: 0.98-0.99), P<.001 and

0.99(0.97-0.99), P<.001] for the corneal curvature and anterior chamber depth, respectively. With this very high level of agreement, the Hirschberg ratio of a subject would not have been significantly different regardless of which eye was used in the prism-based technique; 2) subjects in the LVPEI cohort includes myopes who were not optically corrected during the data collection process while those at UU were corrected with soft contact lenses (Table 2). While this could affect accurate fixation of targets in theory, it has been reported that blur from moderate levels of uncorrected refractive errors has minimal impact on fixation accuracy, and rather that fixation target characteristics are critical in determining accuracy and stability[28, 29]; 3) the two study centers used different technologies to obtain measures of corneal curvature and anterior chamber depth in subjects (Table 2). Although the individual instruments have been shown to produce accurate and repeatable measures of the two ocular biometric parameters,[22, 30-32] we are unable report on the agreement between these instruments, even though they produce overlapping results. However, despite these differences in protocols, there was a general similarity in the results in that the corneal curvature and anterior chamber depth results were similar in the two cohorts, the prism-based technique demonstrated the least repeatability and the theoretical technique demonstrated the best repeatability amongst the three protocols tested. These indicate that results obtained in this study are not limited to the specific protocol being followed but it reflects a more general trend of one calibration protocol being of lesser utility than others in obtaining accurate and repeatable estimates of gaze position using 1stPurkinje image-based eye trackers.

In conclusion, the study demonstrates the existence of inter-and intra-subject variance of the Hirschberg ratio in all three methods employed to convert the millimetre separation between the 1st Purkinje image and pupil center into angular units. Consequently, using the population-average Hirschberg ratio may lead to inaccurate estimates of gaze position as shown in the present study. The prism-based and theoretical techniques both demonstrated relative inaccuracies to the eccentric viewing technique. However, the prism-based technique showed the poorest repeatability. In comparison, the theoretical and eccentric viewing techniques demonstrated better repeatability of Hirschberg ratio.

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Figure legends

Figure 1: Raw data of eye position recorded by the PowerRef 3™ plotted as a function of time in the in eccentric viewing calibration (panel A) and prism-based calibration techniques (panel B) for one representative emmetropic subject. Positive and negative values in the y-axis indicate leftward and rightward gaze rotations, respectively, in the eccentric viewing calibration technique. Positive and negative values in the y-axis indicate the effect of base-out and base-in prisms, respectively, in the prism-based calibration technique. The right eye was occluded in the eccentric viewing calibration technique and therefore the instrument did not record any data in this eye (panel A). The right eye fixated on a distant target while prisms were placed before the left eye that was occluded using an infrared transmitting filter in the prism-based calibration technique (panel B). Mean (95% CI) of the eye position data recorded by the PowerRef 3™ plotted as a function of eccentric stimulus position (panel C) and as a function of prism power (panel D) for the same representative subject. The solid line through the data represents the best-fit linear regression equation while the dashed line indicates the unity line.

Figure 2: Box and Whisker plots of the baseline Hirschberg ratios obtained using the eccentric viewing, prism-based, and theoretical techniques (panel A) and the anterior chamber biometric properties of the eye (panel B) for calculating the Hirschberg ratio using the theoretical technique in the UU cohort. The solid horizontal line within the box indicates median value, lower and upper edges of the box indicate the 25^{th} and 75^{th} interquartile range and lower and upper whiskers show the 1^{st} and 99^{th} quartiles. The squares represent individual data points.

Figure 3: Bland-Altman type plots show the agreement between the Hirschberg ratios obtained using the three calibration techniques in the UU cohort. Panel A shows the agreement between the prism-based and eccentric viewing techniques, panel B shows the agreement between the prism-based and theoretical techniques and panel C shows the agreement between the eccentric viewing and theoretical techniques. The solid black lines in all panels indicate the mean absolute difference between the two measurements while the dashed black lines indicate the 95% limit of agreement. The mean difference (MD) and the limits of agreement (LOA) obtained for each comparison is noted in the figure panel.

Figure 4: Bland-Altman type plots of repeatability of three calibration techniques in the UU cohort. Panel A shows repeatability of the eccentric viewing technique, panel B shows repeatability of the prism-based technique and panel C shows repeatability of the theoretical technique. The solid black

lines in all panels indicate the mean difference between the two measurements while the dashed black lines indicate the 95% limit of agreement. The mean difference and the limits of agreement obtained for each comparison is noted in the figure panel.

Figure 5: Box and Whisker plots of the baseline Hirschberg ratios obtained using the eccentric viewing, prism-based, and theoretical techniques (panel A) and the anterior chamber biometric properties of the eye (panel B) for calculating the Hirschberg ratio using the theoretical technique in the LVPEI cohort. The solid horizontal line within the box indicates median value, lower and upper edges of the box indicate the 25th and 75th interquartile range and lower and upper whiskers show the 1st and 99th quartiles. The squares represent individual data points.

Figure 6: Bland-Altman type plots show the agreement between the Hirschberg ratios obtained using the three calibration techniques (panels A – C) and the repeatability of the prism-based technique (panel D) in the LVPEI cohort. The solid black lines in all panels indicate the mean absolute difference between the two measurements while the dashed black lines indicate the 95% limit of agreement. The mean difference and the limits of agreement obtained for each comparison is noted in the figure panel.

Table 1: Characteristics, advantages, and disadvantages of the three gaze position calibration techniques. PI – 1st Purkinje image, PC – Centre of entrance pupil, AC- Anterior Chamber and HR – Hirschberg Ratio.

Technique	Characteristics	Advantages	Disadvantages
Eccentric-Viewing	• Subject fixates on targets placed at known angular eccentricities. • Separation between PI and PC for each target eccentricity is measured. • Reciprocal of slope of linear regression fit of measured separation between PI and PC against target eccentricity gives HR. • Standard calibration routine in most eye trackers.	• Easy to perform in adults and healthy subjects. • Requires minimal technology.	• Assumes that subject is fixating accurately at the expected target location. • Unsteady head position can affect measurement. • Resistance to monocular occlusion in some subjects can make data collection difficult. • Data acquisition can be difficult in uncooperative subjects like infants and children.
Prism-based	• Involves the use of prisms of known base powers to create a separation between PI and PC while one eye is occluded with IR filter • A reciprocal of the slope of the linear regression fit of the separation between the PI and PC against prism power gives the HR.	• Requires minimal technology (loose prisms in a trial case can be used). • Requires minimal participation from subject. • Can be used in infants and children.	• Can be time consuming (e.g. if reflections are present during measurements). • Resistance to monocular occlusion in some subjects can make data collection difficult. • Chance of binocular fusion if monocular occlusion technique is inappropriate.
Theoretical	• HR is derived from anterior chamber biometry of the eye (i.e. corneal curvature and AC depth). • Corneal curvature and AC depth converted into HR using a formula described by Brodie.[7]	• HR can be obtained more quickly than other two techniques. • Less reliant on participant's cooperation. • Less reliant on gaze changes	• Dependency on the availability of technology for biometric measures. • Accuracy of HR estimates depends on accuracy and repeatability of the biometric device.

Table 2: Subject characteristics, experimental set-up and data collection protocols used at the University of Ulster (UU) and at the L V Prasad Eye Institute (LVPEI).

Subject details	**UU**	**LVPEI**
Sample size	28	30
Age	18 - 40	18 – 40
Refractive error details		
Overall	†0.44 D (25^{th} -75^{th} IQR: -0.13-0.88D)	0.00 D (25^{th}-75^{th} IQR: -2.25 – 0.00D)
i. Myopia	CL corrected, n=9	Uncorrected, n=10
ii. Emmetropia	n=19	n=20
Experimental set up		
Eccentric viewing		
i. Target used	6Maltese crosses	6 LEDs
ii. Viewing distance	2m	3m
iii. Visual angle subtended	$\pm 12^{\circ}$ (in 4°steps)	$\pm 15^{\circ}$ (in 5° steps)
iv. Fixating eye	Left eye	Left eye
v. Illumination	Dim	Dim
Prism-based		
i. Prism range used	4 – 16ΔD	4 – 25ΔD
ii. Vertex distance	10 – 14mm	10 – 14 mm
iii. IR filter used	Optcast Filter NT3-953	Optcast Filter NT3-953
iv. Fixating eye	Dominant eye	Left eye
Theoretical		
CC and AC depth Instrument used	Zeiss IOL Master™	Wavelight® Oculyzer™ II diagnostic device
Data collection protocol used		
Eccentric-viewing		
i. Intra-subject repeatability	Yes (n=28)	No
Prism-based		
i. Intra-subject repeatability	Yes (n=28)	Yes (n=30)
Theoretical		
Intra-subject repeatability	Yes (n=28)	No

† Median refractive error for all participants

Table 3: Repeat measures of median regression slopes, and Hirschberg ratios (HRs) (full range) for three calibration technique**s** and Intraclass Correlation Coefficient (ICC) test of agreement between baseline and repeat measures. Baseline values represent first visit measurements. Intra-subject variability in each technique was calculated from the MEAN difference (95% Limit of agreement) between the baseline and repeat measurements.

	Regression slope (unitless)	**Median (full range) HR (°/mm)**	**Intra-subject variability** Mean difference (95% limits of agreement)	**Intraclass correlation Coefficient (ICC) rho (95% CI)**
	UU			
Eccentric viewing				
Baseline	0.99	11.90 (10.61 – 14.63)	0.05 (-0.30 to 0.40)	0.99 (0.98-0.997)
Repeat measurement	0.99	11.94 (10.28 – 14.29)	(t=1.63, P=.12)	P<.001
Prism-based				
Baseline	0.89	13.30 (11.47 – 16.93)	0.09 (-1.91 to 2.08)	0.88 (0.74-0.944)
Repeat measurement	0.87	13.59 (11.34 – 17.83)	(t=0.44, P=.66)	P<.001
Theoretical				
Baseline	N/A	11.43(9.84 – 13.44)	0.04 (-0.20 to 0.28)	0.99 (0.99-0.998)
Repeat measurement	N/A	11.45(9.82 – 13.18)	(t= 1.93, P= .07)	P<.001
LVPEI				
Eccentric viewing				
Baseline	1.04	11.41 (9.52 –14.94)	-	
Repeat measurement	-	-		
Prism-based				
Baseline	0.97	12.16 (10.54 – 15.45)	0.08 (-1.70 to 1.90)	0.83 (0.64 - 0.92)
Repeat measurement	0.99	11.92 (10.54 – 14.57)	(t=0.49, P=.63)	P<.001
Theoretical				
Baseline	N/A	11.72 (10.14 – 13.25)		
Repeat measurement	-	-	-	

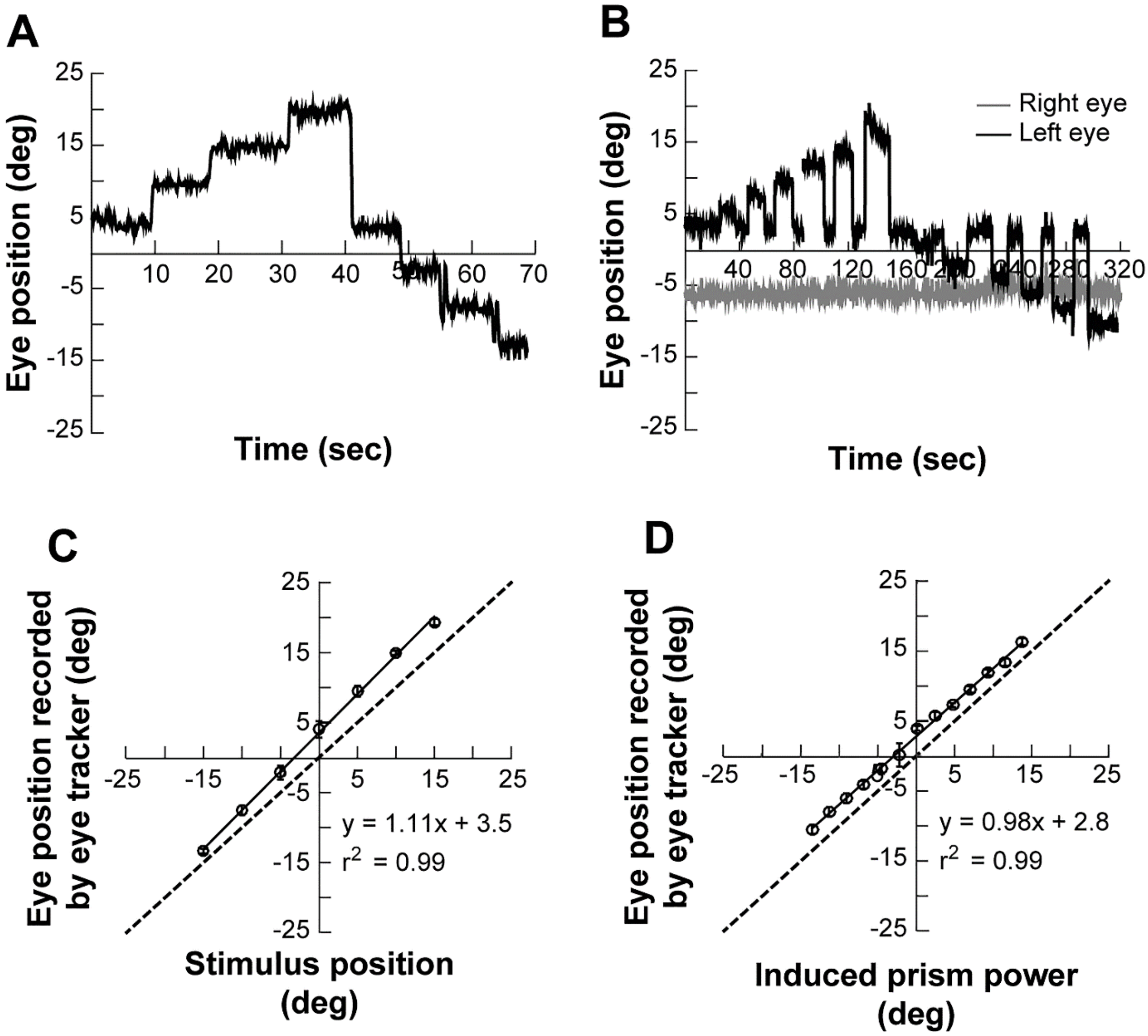
t represents paired t test of the mean difference between baseline measures and repeat measurement, and P represents the statistical significance.

Table 4. Results of mean signed and mean absolute difference between the three techniques. The mean signed difference was computed using one-way ANOVA, with post-hoc test employing Scheffe method. P-values represent probability of mean difference being statistically significantly different from zero.

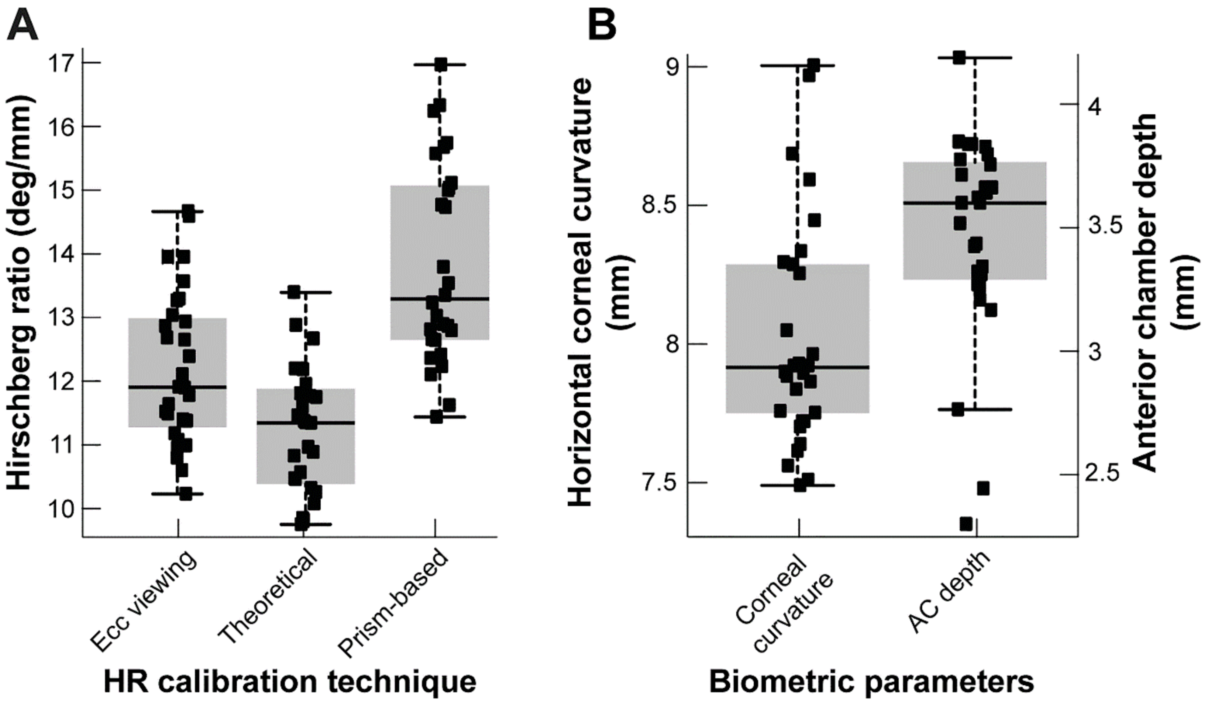
	Mean difference (signed) Mean °/mm (95% LOA)	Mean difference(absolute) Mean °/mm (95% LOA)
UU		
Prism-based vs Eccentric viewing technique	-1.63 (-1.17 to 4.43) P<.001	1.87 (-0.27 to 4.01) P<.0001
Prism-based vs Theoretical technique	-2.54 (-0.32 to 5.40) P<.001	2.54 (-0.32 to 5.40) P<.0001
Eccentric viewing vs Theoretical technique	-0.91 (-1.40 to 3.22) P<.024	0.98 (-1.22 to 3.18) P<.001
LVPEI		
Prism-based vs Eccentric viewing technique	-0.77 (-1.58 to 3.1) P<.03	1.11 (-0.63 to 2.85) P<.0001
Prism-based vs Theoretical technique	-0.67 (-0.98 to 2.30) P<.06	0.91 (-0.19 to 2.01) P<.0001
Eccentric viewing vs Theoretical technique	0.10 (-1.73 to 1.53) P=.93	0.57 (-0.61 to 1.75) P<.0001

Negative values observed in the 95% LOA for the mean absolute difference column is due to the mean difference data being skewed, although individual data points were all positive (see Figures 3A-C and 6A-C).

Figure 1A-D


A
Eye position (deg)
Time (sec)
B
Right eye
Left eye
Eye position (deg)
Time (sec)
C
Eye position recorded by eye tracker (deg)
y = 1.11x + 3.5
r2 = 0.99
Stimulus position (deg)
D
Eye position recorded by eye tracker (deg)
y = 0.98x + 2.8
r2 = 0.99
Induced prism power (deg)

523 Figure 2A-B



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525

Figure 3A-C

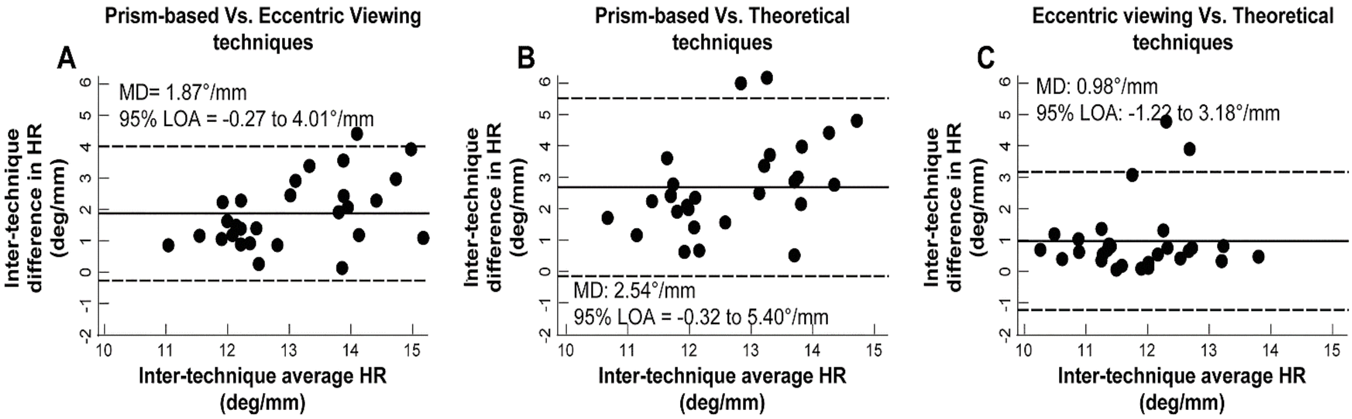


Figure 4A-C

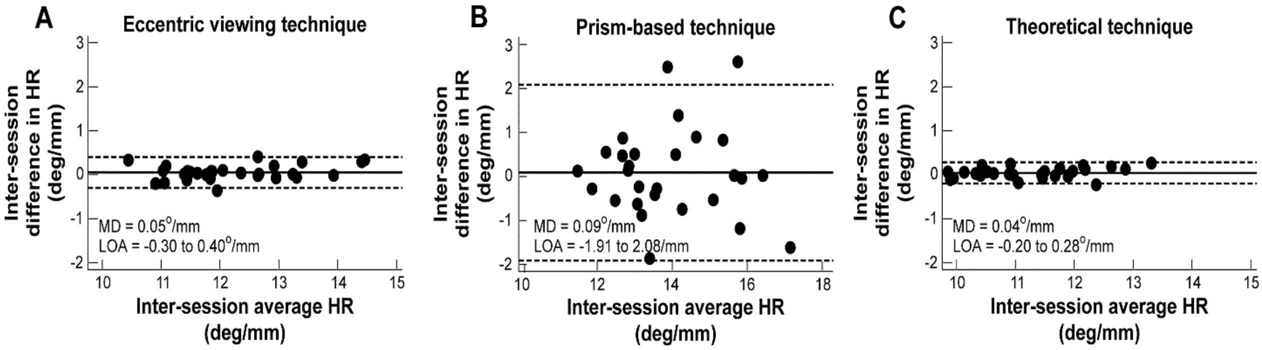


Figure 5A-B

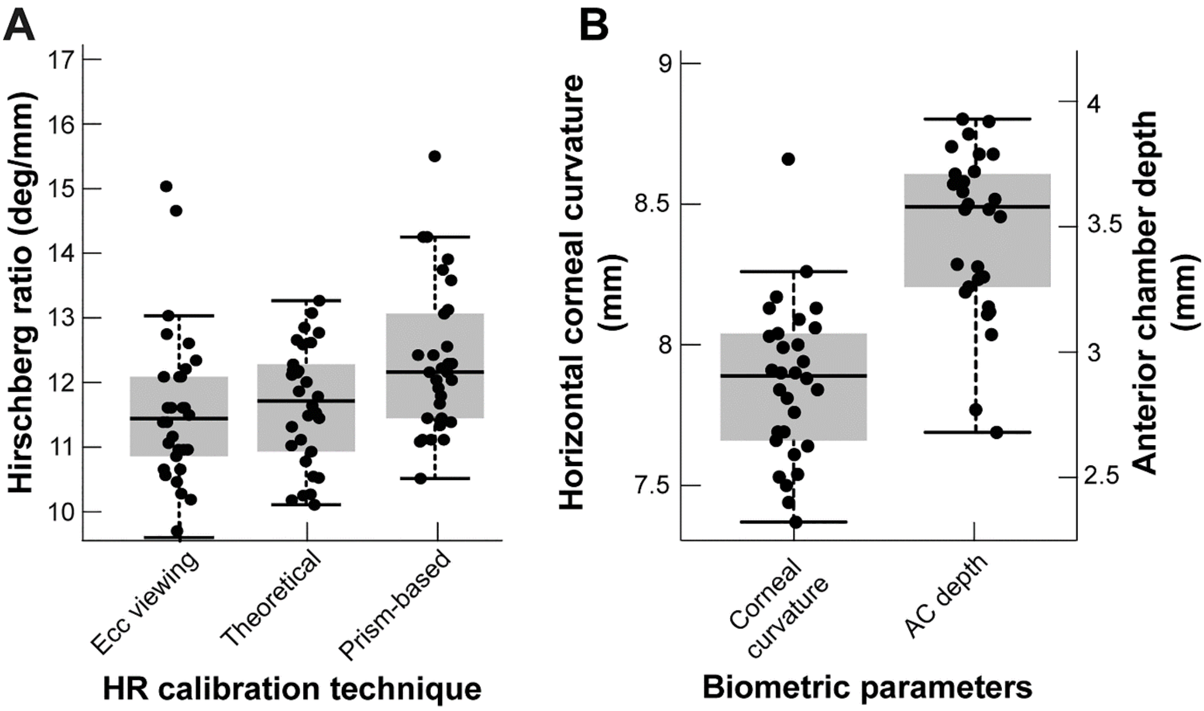
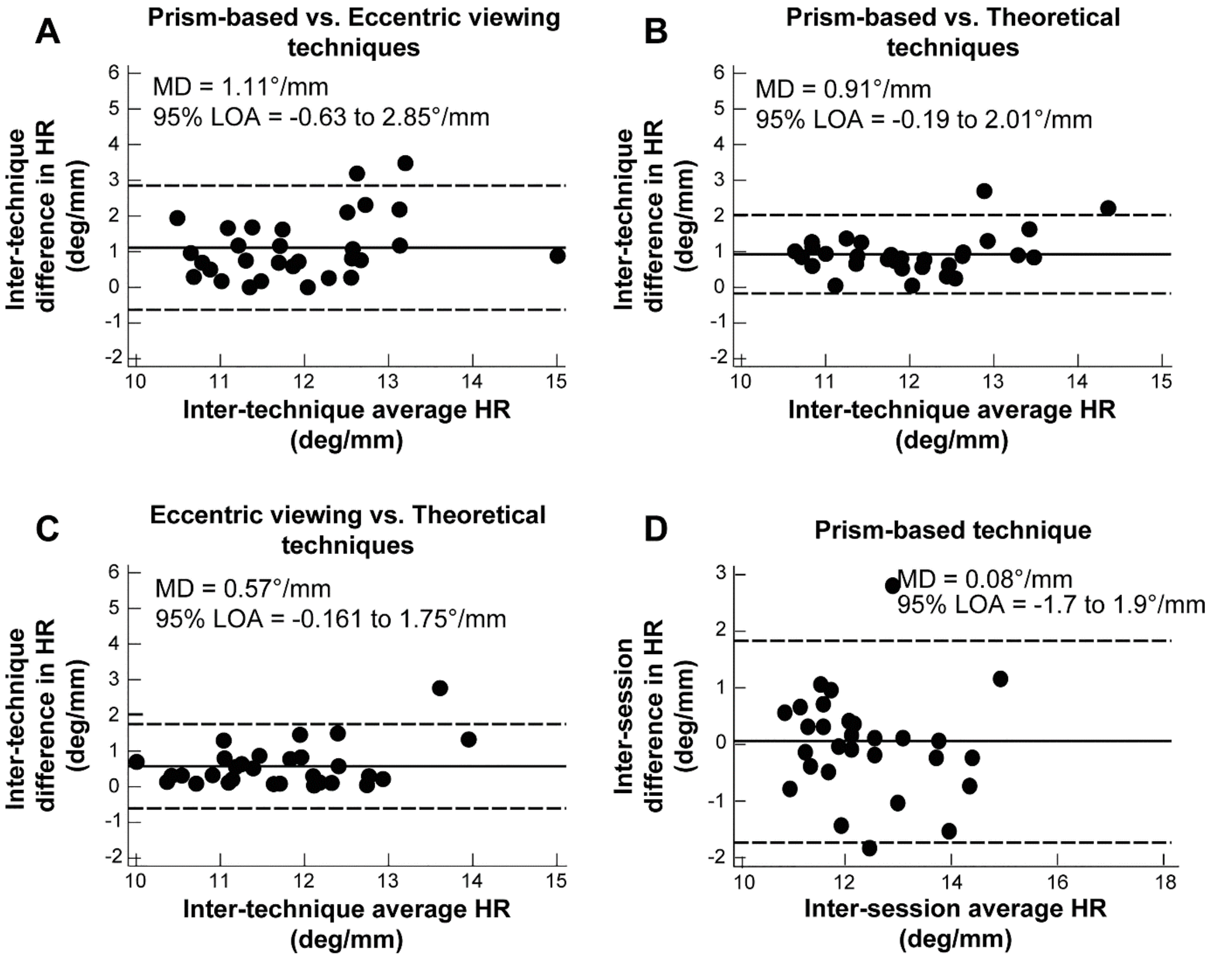

A
Hirschberg ratio (deg/mm)
17
16
15
14
13
12
11
10
Ecc viewing
Theoretical
Prism-based
HR calibration technique
B
Horizontal corneal curvature (mm)
9
8.5
8
7.5
Anterior chamber depth (mm)
4
3.5
3
2.5
Corneal curvature
AC depth
Biometric parameters

Figure 6A-D


A
Prism-based vs. Eccentric viewing techniques
MD = 1.11°/mm
95% LOA = -0.63 to 2.85°/mm
Inter-technique difference in HR (deg/mm)
Inter-technique average HR (deg/mm)
B
Prism-based vs. Theoretical techniques
MD = 0.91°/mm
95% LOA = -0.19 to 2.01°/mm
Inter-technique difference in HR (deg/mm)
Inter-technique average HR (deg/mm)
C
Eccentric viewing vs. Theoretical techniques
MD = 0.57°/mm
95% LOA = -0.161 to 1.75°/mm
Inter-technique difference in HR (deg/mm)
Inter-technique average HR (deg/mm)
D
Prism-based technique
MD = 0.08°/mm
95% LOA = -1.7 to 1.9°/mm
Inter-session difference in HR (deg/mm)
Inter-session average HR (deg/mm)